

silanized, autoclaved 30 ml Corex tubes with 15 ml QF buffer (50°C). Isopropanol (10.5 ml) was added to each sample, the tubes covered with parafilm and mixed by repeated inversion until the DNA precipitated. Samples were pelleted by centrifugation in the SS-34 rotor at 15,000 rpm for 10 minutes at 4°C. The pellet location was marked, the supernatant discarded, and 10 ml 70% ethanol (4°C) was added. Samples were pelleted again by centrifugation on the SS-34 rotor at 10,000 rpm for 10 minutes at 4°C. The pellet location was marked and the supernatant discarded. The tubes were then placed on their side in a drying rack and dried 10 minutes at 37°C, taking care not to overdry the samples.

After drying, the pellets were dissolved into 1.0 ml TE (pH 8.5) and placed at 50°C for 1-2 hours. Samples were held overnight at 4°C as dissolution continued. The DNA solution was then transferred to 1.5 ml tubes with a 26 gauge needle on a tuberculin syringe. The transfer was repeated 5x in order to shear the DNA. Samples were then placed at 50°C for 1-2 hours.

(2) Quantitation of genomic DNA and preparation for gene amplification assay:

The DNA levels in each tube were quantified by standard A_{260} , A_{280} spectrophotometry on a 1:20 dilution (5 μ l DNA + 95 μ l ddH₂O) using the 0.1 ml quartz cuvettes in the Beckman DU640 spectrophotometer. A_{260}/A_{280} ratios were in the range of 1.8-1.9. Each DNA sample was then diluted further to approximately 200 ng/ml in TE (pH 8.5). If the original material was highly concentrated (about 700 ng/ μ l), the material was placed at 50°C for several hours until resuspended.

Fluorometric DNA quantitation was then performed on the diluted material (20-600 ng/ml) using the manufacturer's guidelines as modified below. This was accomplished by allowing a Hoeffer DyNA Quant 200 fluorometer to warm-up for about 15 minutes. The Hoechst dye working solution (#H33258, 10 μ l, prepared within 12 hours of use) was diluted into 100 ml 1x TNE buffer. A 2 ml cuvette was filled with the fluorometer solution, placed into the machine, and the machine was zeroed. pGEM 3Zf(+) (2 μ l, lot #360851026) was added to 2 ml of fluorometer solution and calibrated at 200 units. An additional 2 μ l of pGEM 3Zf(+) DNA was then tested and the reading confirmed at 400 \pm 10 units. Each sample was then read at least in triplicate. When 3 samples were found to be within 10% of each other, their average was taken and this value was used as the quantification value.

The fluorometrically determined concentration was then used to dilute each sample to 10 ng/ μ l in ddH₂O. This was done simultaneously on all template samples for a single TaqMan plate assay, and with enough material to run 500-1000 assays. The samples were tested in triplicate with Taqman™ primers and probe both B-actin and GAPDH on a single plate with normal human DNA and no-template controls. The diluted samples were used provided that the CT value of normal human DNA subtracted from test DNA was \pm 1 Ct. The diluted, lot-qualified genomic DNA was stored in 1.0 ml aliquots at -80°C. Aliquots which were subsequently to be used in the gene amplification assay were stored at 4°C. Each 1 ml aliquot is enough for 8-9 plates or 64 tests.

Gene amplification assay:

The PRO polypeptide compounds of the invention were screened in the following primary tumors and the resulting Δ Ct values greater than or equal to 1.0 are reported in Table 9 below.

Table 9
ΔCt values in lung and colon primary tumor and cell line models

Tumor or Cell Line	PRO 213-1	PRO 237	PRO 324	PRO 351	PRO 362	PRO 615	PRO 531	PRO 853	PRO 1017	PRO 618	PRO 772	PRO 703	PRO 792	PRO 474	PRO 274	PRO 381	PRO 717	PRO1330 md	PRO1449
LT-1	1.60	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	1.60	---
LT-1a	1.24	1.04	---	---	---	---	1.70	---	1.785	---	1.33	1.22	1.16	1.94	---	---	---	1.24	---
LT2	---	---	---	---	1.39	---	---	---	---	---	---	---	---	---	---	---	---	---	---
LT3	1.51	1.74	---	---	---	1.31	1.95	---	2.38	1.03	1.11	1.77	1.10	2.55	---	---	---	1.51	---
						1.55	1.24						1.52						
LT4	2.26	---	---	---	1.00	---	1.46	---	---	---	---	---	---	---	1.24	---	---	2.26	---
LT6	1.56	1.16	---	---	---	1.00	2.07	---	2.80	---	1.07	1.15	1.81	2.10	---	---	---	1.56	---
													2.28						
LT7	2.45	1.44	---	---	---	1.09	---	---	1.12	---	---	1.44	---	1.06	---	---	---	2.45	---
						1.03													
LT9	1.24	---	---	1.19	---	1.04	1.10	---	2.74	1.39	1.62	---	1.99	2.56	---	---	---	1.24	---
						1.14				1.11				2.59					
LT10	---	1.20	---	1.06	1.69	1.18	1.96	---	3.52	1.29	1.46	1.48	2.00	2.63	---	---	---	---	---
						1.11	1.16			1.29				2.85					
LT11	2.26	---	1.34	1.02	---	1.46	1.79	1.03	1.54	1.84	1.45	1.90	1.20	1.36	---	---	---	2.26	---
	2.85					1.72			2.94			1.83	5.21					2.85	
	2.25					1.27			1.41									2.25	
	1.79					1.25												1.79	
						1.06													

Tumor or Cell Line	PRO 213-1	PRO 237	PRO 324	PRO 351	PRO 362	PRO 615	PRO 531	PRO 853	PRO 1017	PRO 618	PRO 772	PRO 703	PRO 792	PRO 474	PRO 274	PRO 381	PRO 717	PRO1330 and PRO1449
LT12	1.86 4.32 2.59 1.55	--	1.92	--	--	2.08 1.87 1.41 1.50 1.25	1.86 1.86 1.41 1.50 1.25	1.18 3.02 1.82	1.77 3.02 1.82	--	--	1.38 1.62	--	1.64 5.01	--	--	--	1.86 4.32 2.59 1.55
LT13	1.98 2.52 2.38	1.05	--	1.23	--	1.39 1.09 1.03	2.53 2.06 1.31	1.33 2.14 2.03	1.55 2.14 2.03	--	1.18 1.33 1.20	1.33 1.33 1.00	1.03 1.00 4.54 1.14 1.65	--	--	--	7.03	1.98 2.52 2.38
LT15	1.40 1.58 2.69	--	--	1.14	--	1.67 1.47 1.09	2.56 2.95 1.31	1.28 2.01 2.50	2.23 2.01 2.50	--	1.47 1.44	1.45 1.44	1.04 1.86 4.97 1.52	1.35 1.00 4.23 1.32 1.17	--	--	2.71	1.40 1.58 2.69
LT16	1.22 2.77 1.75	1.22	1.63	1.09	--	1.32 1.38	--	1.33 1.77	2.98 1.77	--	--	1.07	--	4.23 1.32 1.17	1.00	--	5.48	1.22 2.77 1.75
LT17	4.58 3.73 5.55	1.07	1.75	1.46	--	1.66 1.59 1.21 1.50 1.13	1.12 1.53 1.21 1.50 1.13	1.21 1.62	2.90 1.62	1.04 1.62	1.42 1.61	1.24 1.61	1.35 1.115 5.45	1.40 5.45	--	--	--	4.58 3.73 5.55
LT18	--	--	--	1.07	--	--	--	--	3.28 1.68	--	--	--	--	5.31	1.61	--	--	--